

Claims

1. A method of amplifying gene expression in a moss plant cell
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1)providing at least a first heterologous nucleic acid
construct comprising at least one heterologous nucleotide
sequence operably linked to a promoter, wherein the said
10 construct is flanked at the 5' end thereof by a first
recombination sequence and is flanked at the 3' end of the
said construct by a second recombination sequence in the same
orientation as the first;

15 2)providing at least a second heterologous nucleic acid
construct comprising at least one heterologous nucleotide
sequence operably linked to a promoter, wherein the said
construct is flanked at the 5' end thereof by said second
recombination sequence and is flanked at the 3' end of the
20 said construct by said first recombination sequence in the
same orientation as the second; and

3)transforming into the moss plant cell at least said first
and said second heterologous nucleic acid construct.
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2. A method according to claim 1 wherein the said at least first
construct and the said at least second construct are co-
transformed into a moss protoplast.

30 3. A method according to claim 1 or claim 2 wherein the said
first construct and the said second construct is comprised of at
least one set of complementary recombination sequences.

4. A method according to any one of the preceding claims wherein the recombination sequences are derived or selected from genomic DNA, cDNA, intron, a non-coding region or an exon or any combination thereof.

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5. A method according to claim 4 wherein the recombination sequence is selected from an intron or non-coding region.

6. A method according to claim 4 or claim 5 wherein the length of the recombination sequences is from 25 to 1000 nucleotides long.

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7. A method according to claim 6 wherein the length of the recombination sequences is from 50 - 650 nucleotides long.

8. A method according to claim 7 wherein the length of the recombination sequences is from 100 - 400 nucleotides long.

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9. An heterologous DNA construct of the invention that comprises in the 5' to 3' direction:

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- 1) an introduced first recombination sequence;
- 2) at least a heterologous nucleic acid sequence of interest comprising a promoter operably linked thereto and optionally a terminator therefor; and
- 3) an introduced second recombination sequence.

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10. An heterologous DNA construct of the invention that comprises in the 5' to 3' direction:

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- 1) an introduced second recombination sequence;
- 2) at least a heterologous nucleic acid sequence of interest comprising a promoter operably linked thereto and optionally a terminator therefor; and
- 3) an introduced first recombination sequence.

11. DNA constructs according to claim 9 and claim 10 wherein the recombination sequences of steps 1) and 3) respectively are complementary to each other and are oriented in the same direction.

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12. A DNA construct according to any of claims 9 to 11 wherein the construct is a linear DNA construct.

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13. A moss cell transformed with at least two complementary DNA constructs according to any one of claims 9 to 12.

14. A moss cell according to claim 13 which is a moss protoplast or a moss protonema cell.

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15. A moss cell according to claim 14 which is derived from *Physcomitrella patens*.

16. Moss protonema tissue comprised of cells transformed with at least two constructs according to any one of claims 9 to 12.

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17. Use of moss protonema cells transformed with DNA constructs according to any one of claims 9 to 12 in the production of protein therefrom.

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18. Use according to claim 17 of moss protonema cells derived or selected from *Physcomitrella patens* that are transformed with at least two DNA constructs carrying at least a set of complementary recombination sequences.